OBSERVATIONS ON THE FINE STRUCTURE OF THE MERISTEM OF ROOT NODULES FROM SOME ANNUAL LEGUMES

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(Plates xi-xxv)

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Synopsis

The fine structure of the meristematic zone of the root nodules of subterranean clover, barrel medic and purple vetch was examined with thin sections of $KMnO_4$ and OsO_4 fixed tissue. The nodule meristematic cell has basically the same ultra-structure as other types of meristematic cells, as described in the literature. The differentiation of the cells produced by the meristem to form the cells invaded by the rhizobia is also described. The fine structure of the nodule husk cells is compared with those of the nodule meristem.

INTRODUCTION

The root nodules of subterranean clover and barrel medic and purple vetch are formed by the differentiation of cells produced by an apical meristem. The cells which differentiate basally in relation to the zone of cell division form the region of the nodule which becomes filled with bacteroids, while cells differentiating terminally and laterally form the husk or nodule cortex, and in this region further differentiation forms the vascular system of the nodule. Development of cells in the bacteroid zone of the nodule has been described (Dart and Mercer, 1963a, 1963b ; 1964).

MATERIALS AND METHODS

Plants of subterranean clover (*Trifolium subterraneum* L. var. Clare) inoculated with the effective *Rhizobium trifolii* str. TAI, and barrel medic (*Medicago tribuloides* Desr. str. 173) inoculated with the *Rhizobium meliloti* strain SU277.1, an effective strain, or SU237, were grown in sand culture in a greenhouse. This latter strain forms nodules which are red for only 3–5 days. A description of these nodule types has been given previously (Dart and Pate, 1959). Nodules from *Vicia atropurpurea* Desf. (purple vetch) formed by the effective *Rhizobium* strain SU331 were also examined. For the effective strains, slices of 1–4 week old nodules were examined ; but for the SU237 strain, slices were taken from nodules both before they became pigmented and during the pigmented phase. The nodule slices were fixed in $KMnO_4$ or OsO_4, stained in uranium acetate, and embedded in araldite. Thin sections were examined in a Siemens Elmiskop I or II.

Full details of techniques have been described previously (Dart and Mercer, 1963).

OBSERVATIONS AND DISCUSSION

The low power electron micrographs (Pls xi ; xii, *a* ; xiii) show the general fine structural features of the nodule meristematic cells. There is a relatively large nucleus usually containing one nucleolus, mitochondria, proplastids, Golgi bodies, endoplasmic reticulum occasionally connected with the nuclear membrane, a ground cytoplasm with many ribosome-like particles, and occasionally " spherosomes " and unidentified vesicular organelles. As can

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be seen, the basic structure is similar to the ultrastructure of other types of meristematic cells (e.g. root apex Whaley *et alii*, 1960 ; Falk, 1962 ; and stem apex Buvat, 1958). This confirms the suggestion made from light microscope observations that " the meristematic cell of the nodule corresponds to a meristematic cell of any growing region " (Fred, Baldwin and McCoy, 1932). Intercellular spaces are not usually present in the meristem, but develop as the cells differentiate (Pl. xi).

Mitochondria

In $KMnO_4$ fixation, the mitochondria are mostly spherical to ellipsoid in shape, and have the usual structure with cristae arising from the inner limiting membrane and a homogeneous matrix between the cristae. Mitochondria from meristematic and newly differentiated cells usually contain a prominent ' vacuole ' in this matrix, superficially similar to the *Rhizobium* nucleoid, and it is tempting to associate this region with mitochondrial deoxyribonucleic acid (e.g. see Nass and Nass, 1963 ; Nass *et alii*, 1965 ; Bell and Mühlethaler, 1964 ; Gibor and Granick, 1964). Occasionally some mitochondria have figure-of-eight shapes, a narrow constriction separating the two lobes—suggesting division by constriction (Pl. xiv, *b* ; Pl. xxiii, *a*). Similar origins for new mitochondria have been proposed by other workers (e.g. Whaley *et alii*, 1960). No large promitochondrion bodies which segment to form mitochondria (Bal and De, 1961 ; Manton, 1962 ; Vesk, Mercer and Possingham, 1965) were found in the nodule meristem. Mitochondria in the differentiated uninvaded cells usually are smaller with fewer cristae than those of the meristem.

Proplastids

These organelles are prominent features of the nodule meristematic cell, and are scattered through the cytoplasm. They are usually bounded by two membranes which enclose a poorly developed internal membrane system and a matrix or stroma. The limiting membrane generally stains more deeply in $KMnO_4$ fixation than any of the other cell membranes. The internal membranes are formed by invagination of the inner limiting-membrane (Pl. xvii, *c* ; Pl. xviii, *a-c*) or from a " prolamellar body ".

In leaf tissue a prolamellar body has been implicated in the formation of the internal plastid membranes (e.g. Mühlethaler and Frey-Wyssling, 1959). In the nodule plastids the ' bodies ' are much smaller and the membrane bounded compartments less organized than those found in leaf tissue (Pl. xviii, *a-c*).

In the proplastids of the nodule, small ($\simeq 60$ Å diameter), electron-dense granules are found. These are distributed through the stroma of the proplastids before, and at the beginning of, starch formation (Pl. xvi, *a* ; Pl. xviii, *e*) but in young plastids with small starch grains they may be arranged in groups (Pl. xv, *b* ; Pl. xvii, *a*, *b*). The particles resemble the phytoferritin described by Hyde *et alii* (1963) as occurring in young bean and pea plant plastids. Bergersen (1963) has also observed small electron-dense granules in soybean nodule plastids. The particles are much more readily resolved in OsO_4 fixation (Pl. xv, *b* ; Pl. xvi, *a*) for in $KMnO_4$ fixation the electron-density of the plastid stroma tends to mask the granules. In older plastids with large starch grains the particles can occasionally be found. The particles are sometimes organized into a tight array from which tubular (?) profiles appear to originate (Pl. xvii, *a*, *b*). Similar ' tubules ' often run between the normal, internal plastid membranes and the inner limiting membrane of the plastid (Pl. xvii, *a*, *d*, *e* ; Pls xix ; xx ; xxi *b*) but are much less electron dense than the normal plastid membranes. Often there are several of these profiles running roughly parallel to each other, superficially resembling a mitochondrion in outline, although the tubule diameter is much smaller than the profile of a mitochondrion crista in cross section. Serial sections show that the tubules, with the enclosing dense-staining plastid membranes are often organized into a separate, oval-

shaped " compartment " at the edge of the plastid. The membrane bounding the " compartment " in the plastid stroma is not continuous in all sections with the plastid limiting membrane which forms the rest of the " compart- ment " boundary. The " compartment " would appear to be formed by an invagination and folding back of the inner-limiting membrane of the plastid (Pls xix ; xx).

The proplastids in the differentiating cells adjacent to the meristem often have bud-like protrusions (Pls xiv, c ; xv, d ; xviii, e), usually with several membranes running across the " bud ".

The nodule proplastids appear to arise in two related ways—by segmenta- tion of a large membrane bounded body (Pl. xiv, a, b) and by constriction division of an existing proplastid (Pls xiii ; xxiv, b). A similar situation has been described by Vesk, Mercer and Possingham (1965) for the proplastids of leaves of *Zea mays*. Plastids in the nodule meristematic cells rarely contain starch and have few internal membranes, but synthesis and development of these accompanies cell enlargement and vacuolation (Pls xi, a ; xii, b).

In the bacteroid-filled cells plastids become elongated and filled with elongate starch grains (Dart and Mercer, 1963a, 1964) while in the adjacent noninvaded cells the plastids are oval-shaped, and contain three or four large roughly circular, starch grains, but much more plastid stroma remains than in the plastids of the invaded cells (Pl. xvii, c).

As with the mitochondria, proplastids in the nodule meristem often contain, in the stroma, small electron empty ' vacuoles ' crossed by very fine fibrils (Pl. xiv, a). The similarity between these areas and the bacterial nucleoid has been remarked on by others (e.g. Ris and Plaut, 1962), the implication being that these are the deoxyribonucleic acid-containing regions in the plastid (see Gibor and Granick, 1964 ; Gunning, 1965).

Golgi Bodies

Several Golgi bodies are usually present in each thin section of the meristematic cell and each consists of a varying number of flattened discs. Occasionally in $KMnO_4$ fixation, the membrane bounding each disc can be resolved into a unit membrane (Robertson, 1960). Often the rims of the discs are enlarged into vesicular structures which form a sequence ranging from a slightly inflated periphery to large sacs, some of which apparently bud off to form small single membrane bounded vesicles in the cytoplasm, as described for other cells (Whaley *et alii*, 1960, 1964). As in other plant cells (e.g. Whaley and McIlenhauer, 1963), these Golgi vesicles appear to be associated with cell plate and primary wall formation.

Ground Cytoplasm

In $KMnO_4$ fixation the ground substance is a homogeneous granular matrix, while in OsO_4 fixation electron-dense, 120–150 Å diameter ribosome-like particles are found, dispersed through the cytoplasm and associated with the endoplasmic reticulum (Pls xii, a ; xiii ; xv, a ; xvi, a). Surface views of the reticulum (Pl. xiii) show the ribosomes may be organized in a spiral or a linear array with some 8–12 ribosomes per unit (polyribosome ?). The relative concentration of ribosomes in the cytoplasm decreases as the cells differentiate and vacuolation begins. A marked increase in ribosome numbers (mostly free in the cytoplasm) occurs following infection thread invasion and subsequent release and dispersal of the *Rhizobium* cells through the host cytoplasm. The endoplasmic reticulum system is rather sparse in the meristematic cells and is mostly plate-like and generally granular. The amount of endoplasmic reticulum appears to be even less in differentiating cells (Pl. xii, b) before *Rhizobium* invasion. Small spherosome-like bodies (see Frey-Wyssling *et alii*, 1963 ; Drawert and Mix, 1962) are sometimes found in the nodule meristematic cells but are more frequently seen in the differentiating cells. These bodies

have an electron-dense granular composition, and are surrounded by a dense staining membrane. Sometimes an electron-empty region is found in the centre of these bodies (Pl. xviii, *e*). Similar bodies have been found in maize and rye root meristem cells (Whaley *et alii*, 1960 ; Fabergé and Lewis, 1962). Occasionally other unidentified inclusions resembling a sack of tiny vesicles are found (Pl. xxii) in both $KMnO_4$ and OsO_4 fixation, as well as small vesicles with a single membrane enclosing a homogeneous ground substance (Pl. xxii, *a*). The former are closely associated with the endoplasmic reticulum and similar bodies are found next to the cell wall enclosed by the plasma membrane (Pl. xxii, *b*, *d*). This suggests that the bodies may be involved in transport of materials from the endoplasmic reticulum to the cell wall. Jensen (1963) has recently reported that similar bodies in cotton synergids are specialized endoplasmic reticulum vesicles. Occasionally membrane profiles of what is presumably endoplasmic reticulum contain a dense inclusion (Pl. xxii, *a*).

Nucleus

The interphase nucleus occupies a major proportion of the volume of the nodule meristematic cell. In $KMnO_4$ fixation the nucleus is clearly bounded by two unit membranes that are usually closely appressed to each other and OsO_4 fixation shows that the outer membrane is studded with ribosomes. Nuclear pores and larger gaps are occasionally present and there are often several connections between the nuclear envelope and endoplasmic reticulum (Pl. xi).

In early interphase cells, gaps much wider than the normal nuclear pore are often present in the nuclear envelope. Similar large gaps occur in newly invaded, differentiated nodule cells. In both these cell types ribosome synthesis is active at this stage as well as the synthesis of new cytoplasmic proteins. Woodard *et alii* (1961) have shown that in pea root meristem cells there is a rapid synthesis of ribonucleic acid in early interphase. The large gaps in the nuclear envelope would permit rapid transfer of ribosomes to the cytoplasm if in fact ribonucleic acid and protein are organized into ribosomes in the nucleolus (see Bonner and Huang, 1962). Little structure is observable in the interphase nucleoplasm with $KMnO_4$ fixation but occasionally denser granulation, presumably corresponding to chromatin material, can be seen, while the nucleolus appears as a more electron-dense area usually circular in outline. In OsO_4 fixation followed by uranyl acetate staining considerable structure can be seen in the nucleus. Several areas of dense staining materials with an overall granular appearance are present. These dense, presumably euchromatin areas have an irregular outline and are bounded by an electron-lucent nucleoplasm containing dispersed fibrillar material (Pls xii, *a* ; xv, *a*).

The nucleolus is clearly defined in OsO_4 fixation with uranium acetate post staining. It is basically more electron-dense than the surrounding nucleoplasm and chromatin. Occasionally the nucleolus is haloed by an area free of electron-dense material (Pl. xii, *a*) but, as Lafontaine (1958) observed, there are often places where chromatin and nucleolus merge together (Pl. xv, *a*). The nucleolus itself contains tightly packed 130–150 Å diameter granules. The nucleolus often has a relatively electron-lucent core and in the granular cortex there often appears to be filamentous material $\cong 100$ Å wide.

Cell Plate Formation in the Meristem

Following cell division the nuclear membrane often has large gaps near the region of cell plate formation. The new cell plate begins as a collection of vesicular material lined by endoplasmic reticulum and phragmosomes, between the two telophase nuclei. Vesicle coalescence, and consolidation of the electron-lucent material within the new vesicle aggregates, mark the beginning of wall synthesis which continues centripetally (Pl. xi, *b*). In the initial stages of new wall synthesis there are several intercellular endoplasmic

reticulum connections. The pattern of cell division parallels closely that outlined by Porter and Caulfield (1958) and Porter and Machado (1960) for onion root-tip cells, and Whaley *et alii* (1960) for maize root meristematic cells. Towards the end of cell plate formation, evenly dispersed electron-dense material is deposited at the centre of the new wall. As in cell plate formation this zone (middle lamella ?) then consolidates and expands centripetally.

Differentiation of the nodule meristematic cell

After division has ceased the newly-formed cells undergo differentiation to form the husk and bacteria-filled zone of the nodule. The continuous differentiation of the cells and the continuous infection of the differentiated cells maintain a zone, generally three cells wide, between the meristem and the zone of infection.

Vacuolation of the cytoplasm is the first change, associated with differentiation of the nodule cells. Various theories have been proposed for the origin of vacuoles in plant cells, and in electron micrographs of the nodule meristem, profiles of vacuoles can be found which are consistent with most of these views. The most usual '' method '' of vacuole formation observed in the nodule cells was that described by Mühlethaler (1958) where a phase difference becomes apparent in the cytoplasm (Pl. xxiii, *a*) and as this region expands a tonoplast is synthesized *de novo* at the interface in several parts before joining to form the tonoplast observed around fully-developed vacuoles. Buvat (1957, 1958, 1960) and Poux (1962a and b) proposed that vacuoles are initiated by expansion of the two membranes of the endoplasmic reticulum. Membrane profiles consistent with this can be found in differentiating nodule cells (Pls xxii, *a* ; xxiii, *c*) but these may well be plasmolysis figures of vacuoles rather than stages in vacuole formation. Small vesicles are sometimes found associated with the plasmalemma suggesting that pinocytosis might be occurring. Weiling (1961) has suggested that subsequent expansion of pinocytotic vesicles forms a vacuole. In some meristematic cells there are small irregular-shaped, membrane-bounded bodies with phase differences characteristic of vacuoles (Pl. xi). These are presumably the '' pro-vacuoles '' that Whaley *et alii* (1962) and Leech *et alii* (1963) suggest are transformed into true vacuoles. Marinos (1963) claims that the tonoplast in barley shoots is derived from a swelling of the outer Golgi body cisterna but no profiles suggestive of this have been found in the nodule meristem.

The vacuoles in the nodule usually contain a sparsely distributed, electron-dense material. Occasionally dense granular bodies are found in the vacuole and sometimes sharply defined differences in electron-density (phase difference?) exist within the vacuole.

The tonoplast is not always preserved after $KMnO_4$ fixation, but better preservation is obtained with OsO_4 fixation. The tonoplast can be resolved into a unit membrane structure (as defined by Robertson, 1960) with a dark-light-dark profile of overall dimension 90–100 Å. Occasionally after OsO_4 fixation electron-dense material adheres to the vacuole side of the tonoplast.

Some of the cells which differentiate adjacent to the meristem remain uninvaded by *Rhizobium* cells. A proportion of these uninvaded cells, which usually have a thin layer of peripheral cytoplasm intact, degenerate just before adjacent cells become infected. This involves a loss of the cytoplasmic matrix and disorganization of the usual organelles, leaving the plasmalemma and most of the tonoplast intact. In these cells the membranes are readily resolved (Pl. xvi, *b*)—possibly due to a lack of background cytoplasm obscuring the structure, but could also conceivably be due to a change in the membrane structure itself, induced during the cell degeneration. Occasionally small electron-dense lines cross between the two dense lines of the tonoplast membrane giving the membrane a banded appearance similar to the ' globular ' structure observed in mitochondrial and some cytoplasmic membranes by Sjöstrand

(1963). These 'degenerate' uninvaded cells are thought to be a defence mechanism response of the host cell to restrict invasion by *Rhizobium*. Alternatively, these non-living cells may be functioning as vascular or conducting tissue as has been postulated for degenerate cells in pea cotyledon tissue (Bain and Mercer, 1965).

In meristematic cells, but more frequently in the differentiated, recently invaded cells, the plasmalemma often invaginates, enclosing a system of tightly coiled membrane-bounded tubules and vesicles (Pl. xxi, *b*). These structures resemble the lomasomes observed in fungi (Girbardt, 1961; Moore and McAlear, 1961; Peyton and Bowen, 1963). Invaginations of the plasmalemma are also found, with only a few membrane-bounded vesicles between the plasmalemma and the cell wall similar to the structures observed by Grun (1963) in *Solanum* root meristem cells and by us in barrel medic and subterranean clover root meristem cells (Dart and Mercer, unpublished observations). In some cells a single membrane fragment is sometimes found immediately outside the plasmalemma in the cell wall material (Pl. xvi, *b*) and occasionally membranous elements are found deeper in the wall layers (Pl. xxi, *b*). These membrane fragments may be remains from the deposition of material during cell wall thickening (Wardrop, 1964). An incorporation of small, single membrane-bounded vesicles, with the vesicle membrane fusing with the plasma membrane, also appears to be involved in wall development (Pl. xxii, *b*). In other places the vesicles themselves appear to be incorporated in the wall (Pl. xvi, *b*).

Plasmodesmata are frequently observed between meristematic cells, becoming less so as the cell differentiates with associated cell wall growth. Some of these plasmodesmata are branched (Pl. xviii, *e*) and in some the plasmalemma is observed to evaginate and line the structure so that the plasmalemmas of adjacent cells are contiguous. Some plasmodesmata-like structures which penetrate the cell wall are completely bounded in the wall by a membrane-like structure. These might also be Frey-Wysslings "wall papillae" (1962). Only an outer dense zone with an adjacent electron-empty zone can be resolved, presumably because the inner dense line of the membrane (assuming it is a unit membrane) merges with the electron-dense material enclosed by the "membrane" (Pl. xxi, *a*).

Nodule Husk Cells

Quite a distinct difference is apparent between the cells of the nodule meristem and the large, vacuolated, "protective" cells that enclose the nodule. The husk cells have a very large central vacuole, and a thin layer of cytoplasm containing a few small mitochondria with few cristae, Golgi bodies and segments of endoplasmic reticulum (Pls xxiv; xxv). Plastids are few in number and large in size. Starch formation increases with distance from the meristem. The vacuoles usually contain more stainable material than the vacuoles of cells about to be invaded by infection threads (compare Pl. xxv and Pl. xii, *b*).

The nucleus lies in the thin layer of cytoplasm adjacent to the cell wall and often has a wrinkled appearance (Pl. xxv, *c*). Plasmodesmata connections between the husk cells are prevalent—and usually occur in groups (Pl. xxv), possibly corresponding to apit field. Some of the husk cells lose their cytoplasmic contents, leaving a granular material attached to the cell wall in places.

CONCLUSION

There are no basic differences in fine structure between the nodule meristems of subterranean clover, barrel medic or purple vetch. It can be seen that the ultrastructure of the meristematic cell is very similar to the basic ultrastructure of the root meristematic cell. It seems that meristems have a similar subcellular organization and pattern of activity whether they are "normal" structures or whether they arise as a response to invasion by *Rhizobium*.

B

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EXPLANATION OF PLATES XI–XXV

Plate xi.

a.   Panorama of the meristematic zone of a barrel medic nodule (SU237). Vacuole formation and cell enlargement has commenced in some cells. The single arrow points to a ' provacuolar body ' in a meristematic cell which appears to have a ' tail ' of endoplasmic reticulum. The double arrow points to an ' unknown body ' which is bounded by a single membrane. n—nucleus, v—vacuole. KMnO$_4$ fixation. × 11,000.

b.   Cell plate formation in a meristematic cell of a barrel medic nodule (SU237). Endoplasmic reticulum and small vesicles (Golgi vesicles ?) are closely associated with the new wall. The Golgi bodies (g) appear to be budding off small vesicles. The double arrow points to a major discontinuity in the wall. The small mitochondria have prominent ' vacuoles ' (e.g. arrow). p—phragmosome (?). KMnO$_4$ fixation. × 35,000.

Plate xii.

a.   Meristematic cell of a 7-day-old barrel medic nodule (SU237). The nucleus contains sparsely distributed fibrillar material but around the dense nucleolus (Nu) there is a ' halo ' virtually free of fibrils. In the ground cytoplasm there are free ribosomes and some ribosomes attached to endoplasmic reticulum. The proplastids (p) have shrunk during preparation. OsO$_4$ fixation. × 12,500.

b.   Panorama showing the vacuolating cells adjacent to the meristem in a barrel medic nodule (SU237). Most of the plastids contain starch grains. A few sparsely distributed, plate-like endoplasmic profiles are present. KMnO$_4$ fixation. × 11,000.

Plate xiii.

Portion of a meristematic cell from a barrel medic nodule (SU237) showing the distribution of ribosome-like particles, endoplasmic reticulum, Golgi bodies and mitochondria. Surface views of the endoplasmic reticulum show the ribosomes often grouped (polyribosomes ?) into whorled, rosette arrangements (e.g. arrows). The free ribosomes are also grouped in units (e.g. circles). The plastid (p) at the bottom of the figure appears to be dividing by constriction. The inset shows some of the ribosome-like particles in more detail : some of them are attached to endoplasmic reticulum. OsO_4 fixation. $\times 20,000$. Inset $\times 40,000$.

Plate xiv.

a.   Large proplastid bodies in a newly-invaded cell. One appears to be dividing by constriction (arrow). The proplastids contain small electron empty regions (e.g. double arrow) containing fine fibrils which are reminiscent of a bacterial nucleoid (n). $\times 35,000$.

b.   Irregularly-shaped proplastids are apparently segmenting (see double arrow) in a non-invaded meristematic cell. One of the mitochondria has a figure-of-eight profile suggestive of division by constriction (single arrow). w—cell wall. $KMnO_4$ fixation, barrel medic nodules (SU237). $\times 20,000$.

c.   Shows a small 'bud' on a plastid from a barrel medic nodule (SU277.1). $\times 40,000$.

Plate xv.

a.   Interphase nucleus and part of the cytoplasm of a differentiating cell adjacent to the meristem of a barrel medic nodule (SU277.1). The nucleus contains a prominent nucleolus (nu) and several smaller more diffuse electron dense areas (c—presumably euchromatin). OsO_4 fixation. $\times 20,000$.

b.   Plastid from a vacuolated, non-invaded cell of a subterranean clover nodule fixed in OsO_4. The plastid contains a large, central starch grain and several closely packed arrays of phytoferritin-like particles (e.g. arrow). In the adjacent cytoplasm several ribosome-like particles (r) are present, along with a Golgi body (g). $\times 50,000$.

Plate xvi.

a.   Plastids from a meristematic cell of a subterranean clover nodule fixed in OsO_4. The plastids contain numerous phytoferritin-like particles and several larger, osmiophilic bodies (o). Ribosome-like particles (r) are mostly free in the cytoplasm. $\times 60,000$.

b.   Degenerate non-invaded cell in a barrel medic nodule (SU277.1). The tonoplast (t) and plasmalemma (pl) are resolved into a dense-light-dense profile. An invagination of the plasmalemma (arrow) contains several circular membrane profiles apparently embedded in the cell wall and possibly the remains of vesicular packages of material incorporated in the wall. The double arrow indicates another membrane profile running parallel to the plasmalemma and between it and the cell wall. $KMnO_4$ fixation. $\times 70,000$.

Plate xvii.

a.   Proplastid filled with phytoferritin-like particles. These are aggregated in one portion of the plastid (single arrow). The double arrow points to three small tubular elements attached to the limiting plastid membrane. $\times 60,000$.

b.   Another aggregation of the small, electron-dense, particles with some fine tubules running between the aggregation and the plastid limiting membrane. $\times 35,000$.

c.   Plastid from a non-invaded cell. The plastid containing four large starch grains and two areas where an internal plastid membrane is joined to the limiting membrane by fine tubules (arrows). In Fig. d another plastid has been sectioned closer to the edge and shows several of the tubules running between internal plastid membranes and the limiting membrane. 7a–d $KMnO_4$ fixation, barrel medic nodules (SU277.1). 7c and d. $\times 40,000$.

Plate xviii.

a. and b.   are serial sections of a plastid from a vacuolating barrel medic (SU237) nodule cell. An array of small membrane-bounded compartments in the plastid stroma resembles a ' prolamellar body '. In Fig. c a similar body (arrow) can be seen with a well-developed internal plastid membrane attached. The double arrow indicates the junction of an internal plastid membrane with the peripheral membrane. The internal membrane changes from a plate-like form to a tubule at the junction.

d.   Shows a ' bud ' on a plastid from a barrel medic (SU277.1) nodule (division by constriction ?). Small, electron-dense, phytoferritin-like particles are present in the plastid stroma.

e.   Shows a large proplastid from a vacuolated non-invaded nodule cell. The plastid stroma contains numerous phytoferritin-like particles and the ' bud ' on the right contains numerous circular profiles—apparently small, membrane-bounded tubules cut in cross section. A branched plasmodesmata is shown in more detail in the inset. The plasmalemmas of adjacent cells are contiguous and line the plasmodesmata. s—spherosome-like body. × 50,000. Inset × 130,000. Figs *a–d* × 40,000. KMnO₄ fixation, barrel medic nodules. (*d*—SU277.1 ; *a, b, c, e, f*—SU237).

<center>Plates xix–xx.</center>

Plates xix and xx are serial sections (in sequence) of parts of two plastids from a recently invaded cell in a purple vetch nodule.

Plate xix, *f* is oriented about 90° to Plate xix, figs *a–e*. The figures illustrate an arrangement (arrows) of small tubules and plastid membranes. Plate xx, *i* is the same section as Plate xx, *f* showing the location of the tubules within the plastid. Adjacent mitochondria (m) show that the membranes of the plastid inclusion have a different appearance from the mitochondria cristae (e.g. arrow). KMnO₄ fixation. Pl. xix, *a–h*, Pl. xx, *a–h* × 40,000 ; Pl. xx, *i* × 20,000.

<center>Plate xxi.</center>

a.   Shows numerous plasmodesmata-like fragments in the cell wall of an uninvaded, vacuolated cell of a subterranean clover nodule. One of the fragments (arrow) is apparently completely bounded by a membrane—presumably the plasmalemma. KMnO₄ fixation. × 60,000.

b.   Recently-invaded cell in a barrel medic nodule (SU237). The plasmalemma invaginates to enclose a lomasome-like body (l) containing a tightly coiled system of membranes. Membrane envelope synthesis is almost completed around an adjacent *Rhizobium* cell. The inset shows the lomasome-like body at higher magnification. KMnO₄ fixation. × 40,000. Inset × 100,000.

c.   Portion of three differentiating cells adjacent to the meristem. Narrow tubular elements are present in the cell wall adjacent in the middle lamella region and adjacent to a small intercellular space. The arrow indicates where a cristae of a mitochondrion has been sectioned tangentially showing the circular plate-like profile of the cristae. The endoplasmic reticulum is closely associated with the cell wall (double arrow). Barrel medic nodule. KMnO₄ fixation. × 60,000.

<center>Plate xxii</center>

a.   Newly invaded cell and two adjacent uninvaded cells in a barrel medic nodule (SU237) with their intercellular space filled with an electron-dense material. The double arrow points to a profile which could be interpreted as the origin of a vacuole by expansion of endoplasmic reticulum. The single arrow points to bodies containing small vesicles and at (i) a similar body appears to be fused to the cell wall. A dense body (c) is apparently enclosed by endoplasmic reticulum. Another unidentified inclusion is present (b), and it consists of a single enclosing membrane and a homogeneous matrix. A similar body is indicated by the double arrow in Fig. *a*. Yet another unidentified organelle (u) is present in this cell. KMnO₄ fixation. × 40,000.

b.   In *b* two of the bodies containing small vesicles (arrows) lie close to the cell wall and at (i) one has fused with the wall. The double arrow indicates a small bulge of the cell wall partly bordered by similar material to that enclosed by the arrowed bodies suggesting that this may be a later stage of incorporation of wall material to that at (i). Three similar bodies are adjacent to the cell wall (i) in Fig. *d*. In Fig. *b* endoplasmic reticulum profiles and small single-membrane-bounded vesicles are present close to the wall in much greater ' concentration ' than in the rest of the cytoplasm suggesting that they also may have a role in cell wall development.

c.   Portion of a meristematic cell from a barrel medic nodule showing the unidentified bodies (ub) with the single limiting membrane enclosing a number of small vesicles. Similar bodies can also be found after OsO₄ fixation. g—Golgi cisternae. *a–d*, KMnO₄ fixation. *b* × 25,000 ; *c* × 40,000 ; *d* × 50,000.

<center>Plate xxiii.</center>

a.   A ' phase difference ' (v) is apparent in the cytoplasm of a cell from a barrel medic nodule meristem (SU237). It is suggested that this is the first stage in vacuole formation. The arrow indicates a mitochondrion with a figure-of-eight profile suggestive of division by constriction. × 30,000.

b.   A cytoplasmic bridle crosses the vacuole of a differentiating cell in a barrel medic nodule (SU237). The bridle contains a mitochondrion and some endoplasmic reticulum (er). KMnO₄ fixation. × 20,000.

c.   Profile of a vacuole (v) in a subterranean clover nodule with a constricted region where the tonoplast resembles an endoplasmic reticulum profile. The arrow points to other membrane-bounded elements which may be vacuole or expanded endoplasmic reticulum. $KMnO_4$ fixation.   ×25,000.

Plate xxiv.

a.   Young husk cells from a barrel medic nodule (SU237) showing that they contain a similar complement of organelles to the uninvaded, vacuolated cells basal to the meristem. The plastids are relatively large and mitochondria small. The cell wall in places has conspicuous blebs (arrows) which may be the site of incorporation of new wall material. At the top left of the figure an oblique cut through the wall shows several plasmodesmata in cross section (e.g. circle). $KMnO_4$ fixation.   ×10,000.

b.   Plastid from a barrel medic nodule (SU277.1), uninvaded cell, with two small starch grains and two narrow constrictions suggestive of division. $KMnO_4$ fixation.   ×40,000.

Plate xxv.

a.   The cell wall between two husk cells is crossed by several large plasmodesmata. In an adjacent cell (d) the vacuole has collapsed and the cell is degenerating. The arrow points to a coiled membrane fragment. Subterranean clover nodule.   ×12,800.

b.   Shows the thin layer of cytoplasm in some husk cells from a barrel medic nodule (SU237). The cell walls are crossed by several plasmodesmata. $KMnO_4$ fixation.   ×10,000.

c.   Cortex region of a barrel medic nodule (SU237), showing the nucleus (n) and several mitochondria closely appressed to the cell wall. Plasmodesmata are conspicuously grouped in the cell wall.   ×12,800.